Drug Development and Industrial Pharmacy, 25(3), 289–295 (1999)

RESEARCH PAPER

Development and In Vitro Evaluation of Buccoadhesive Tablets Using a New Model Substrate for Bioadhesion Measures: The Eggshell Membrane

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ABSTRACT

For oral delivery of antimicrobial and anti-inflammatory drugs, mucoadhesive tablets based on gelatin/hydroxypropylcellulose (HPC), gelatin/hydroxypropylmethylcellulose (HPMC), and gelatin/sodium carboxymethylcellulose (NaCMC) at different ratios were prepared by direct compression of the mixed powders. Metronidazole and benzydamine were used as model drugs. The in vitro bioadhesive properties, evaluated by a commercial tensile tester, were significantly affected by the model substrate employed, that is, a polypropylene (PP) membrane or a biological membrane (eggshell membrane). The use of the biological substrate seemed to supply more reliable data. All studied formulations showed an erosion-diffusion mechanism of release, anomalous or non-Fickian release, in agreement with the behavior of the swellable systems.

INTRODUCTION

Mouth infections, such as gingivitis and stomatitis, are mostly caused by aerobic and anaerobic microbes and can be treated locally by antimicrobial and anti-inflammatory drugs, usually administered in buccal gel form or as mouthwashes. However, the disadvantage of these delivery systems is that they are easily washed away by the saliva, and the effective drug levels in the mouth are limited to a short period of time; therefore, repeated administrations are often necessary (1,2). To increase the buccal residence time of the drug delivery system, bioadhesive polymers were recently used as buccoadhesive devices that can reversibly adhere to the oral mucosa. These

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preparations can be readily attached to the buccal cavity, retained for a longer period of time, and removed at any time (3).

In previous work, the good adhesive properties of gelatin, employed as a polymeric matrix for buccal release of oxycodone, were reported (4). The aim of the present study was to develop bioadhesive tablets based on different mixtures of gelatin and hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), and sodium carboxymethylcellulose (NaCMC) for local delivery. The addition to these polymeric blends of two drugs, benzydamine (BNZ) and metronidazole (MTZ), commonly used for the treatment of topical infections of the oral cavity, was also evaluated.

Because of the different experimental conditions employed by various workers, the in vitro adhesive properties of polymeric materials often show considerable variability. In this report, two of the several factors that may affect the bioadhesive behavior were considered: the measure apparatus and the model substrate. To evaluate the adhesive forces of the different formulations, a commercial tensile tester was used and, as standard substrates, both a synthetic membrane of polypropylene (PP) and a natural substrate, the eggshell membrane, were investigated.

EXPERIMENTAL

Materials

Benzydamine hydrochloride (BNZ), MTZ (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany), gelatin (commercial type B, Fluka Chemie AG, Switzerland), HPMC (Methocel K15, Colorcon, United States), HPC (Klucel LF PM 95000), NaCMC (Blanose LF; Aqualon, Dusseldorf, Germany), mucin (type II, crude from porcine stomach; Sigma, St. Louis, MO), disodium hydrogen phosphate dihydrate, potassium dihydrogen phosphate, acetonitrile, sodium dodecylsulfate (Merck, Darmstadt, Germany), cellulose acetate membrane filter (Sartorius AG Goettingen, Germany), acetic acid (Riedel-De Haenag, Seelze, Hannover), and demineralized water were used.

The following apparatuses were employed: Sotax AT7 dissolution tester (Sotax Ltd., CH Basel), HP 1090 Series II liquid chromatograph (Hewlett Packard, Avondale, PA), tensile tester equipped with a 20 N load cell (type LRX, Lloyd Instruments, Fareham, UK), mixer mill (Retsch Gmbh, Germany), pH meter (Orion Research, United States), hydraulic press (Perkin-Elmer, Norwalk, CT).

Preparation of Buccoadhesive Tablets

Buccoadhesive tablets were prepared by mixing gelatin with HPMC, HPC, and NaCMC at three different ratios (1:1, 2:1, 4:1) in a mixer mill for 15 min (oscillating frequency 660/min). The obtained mixtures were compressed by a hydraulic press fitted with flat 13-mm punches at a pressure of 3 tons for 1 min. The final tablets had a weight of 200 mg, a diameter of 13 mm, and a thickness of 1.50 mm. Tablets containing both BNZ (5 mg) and MTZ (5 mg) were also prepared as above. The composition of the different formulations is given in Table 1.

Table 1

Composition (mg) of the Buccoadhesive Tablets

Formulations	Gelatin	HPMC	HPC	NaCMC	BNZ	MTZ
MT1	100	100	—	—	—	—
MT2	133	67	—	—	—	—
MT2D	126.7	63.3	—	—	5	5
MT4	160	40	—	—	—	—
KL1	100	—	100	—	—	—
KL2	133	—	67	—	—	—
KL2D	126.7	—	63.3	—	5	5
KL4	160	—	40	—	—	—
BL1	100	—	—	100	—	—
BL2	133	—	—	67	—	—
BL2D	126.7	—	—	63.3	5	5
BL4	160	—	—	40	—	—

Preparation of Biological Substrate

The eggshell membrane was obtained from fresh chicken eggs. After emptying the egg of its contents, the external shell was removed, and the underlying membrane was isolated, taking care to maintain its integrity. Membrane samples were stored at 4°C until use.

In Vitro Determination of Bioadhesion

The force required to separate the sample tablet from a model substrate was recorded using a commercial tensile tester adapted for bioadhesion measurements. A homemade stainless steel jacketed holder and upper metallic support connected to the superior cross-sectional bar bearing the load cell have been designed (Fig. 1). The tablet was fixed to the bottom of the holder by cyanoacrylate adhesive and was wetted with the hydration liquid (10 ml of 2% w/w mucin gel) for 5 min. The synthetic or biological substrate was secured to the upper support of the tensile apparatus and then positioned in contact with the hydrated tablet so that the adhesion bonding could be established. After a preload of 1 N for 5 min (preload time), the tablet and the substrate were pulled apart with a constant extension rate of 0.1 mm/s until a complete rupture of the tablet-substrate bond was obtained. A force-versus-extension diagram was recorded. Using commercial software, the detachment force and the work of adhesion were calculated. All measurements were carried out at 37°C ± 0.5°C.

In Vitro Release Studies

The in vitro release of BNZ and MTZ from buccoadhesive tablets was determined using a dissolution apparatus according to USP method II (paddle). This apparatus consisted of seven polycarbonate vessels placed in a water bath thermostated at 37°C ± 0.5°C and stirred at a rate of 50 rpm. Sink conditions were maintained throughout the study. The tablets were glued to a metallic disk and placed at the bottom of the vessels, thereby allowing drug release only from the upper side and the edges. The dissolution medium was 300 ml of phosphate buffer, pH 6.8 (5.94 g disodium hydrogen phosphate dihydrate and 4.54 g potassium dihydrogen phosphate made up to 1000 ml with demineralized water). Six tablets were examined at the same time. A drug-free tablet, used as a blank, was introduced in the seventh vessel. At fixed time intervals, 5-ml samples were withdrawn and replaced with fresh buffer. Samples were filtered and analyzed by a high-performance liquid chromatography (HPLC) method as described below.

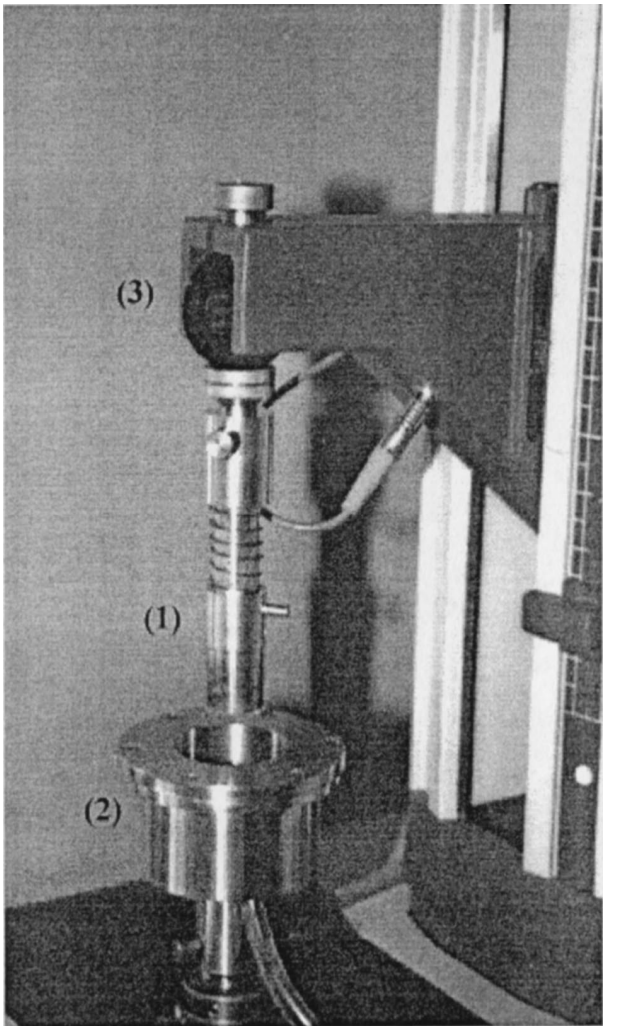

Figure 1. The modified commercial tensile tester for in vitro evaluation of bioadhesion: (1) upper metallic support; (2) stainless steel jacketed holder; (3) load cell.

Analytical Method

To determine BNZ and MTZ concentrations in the dissolution medium, a modified analytical method was employed (5). An HPLC system equipped with a Lichrospher cyano column, 250 mm × 4.0 mm id, average particle size 5 μm (Merck, Darmstadt, Germany) was used. The mobile phase consisted of a mixture of acetonitrile, 0.01 M sodium dodecylsulfate, and acetic acid (60:38.7:1.3 v/v). The aqueous phase was filtered through a

0.45-μm cellulose acetate membrane filter. A flow rate of 1.0 ml/min was used, and the eluent was monitored at 310 nm for BNZ and 320 nm for MTZ using a diode array detector. The reference wavelength was set at 500 nm. The volume of injection was 1.0 μl.

A standard curve was established before each series of measurements with solutions of BNZ and MTZ that ranged from 2 to 20 μg/ml. The standard solutions were prepared by adding 100 μl of appropriate dilutions of a stock solution (1 mg/ml in phosphate buffer) and making up to volume with blank sample in a 5.0-ml volumetric flask. Good linearity, within the considered range, was obtained ($r^2 = .996$), and no interference with the matrix components was observed.

Data Analysis

Analysis of variance (ANOVA) in conjunction with Tukey's test was applied to demonstrate differences in bioadhesion properties of the various polymeric blends. In all statistical tests, $P = .05$ was used as the significance level.

RESULTS AND DISCUSSION

Mucoadhesion Studies

Typical force elongation curves are shown in Fig. 2. The peak value represented the detachment force, and the area under the curve was the work of adhesion. In Table 2 and Table 3, the mucoadhesion results obtained employing eggshell membrane and PP membrane, respectively, are reported. As already indicated by several authors, the bioadhesive properties of polymeric materials are significantly affected by the model mucous membrane employed as the substrate for in vitro bioadhesion measurements. In the literature, different adhesive behaviors of tested materials were reported according to the use of either tissues or mucous membranes of various animals (rat, rabbit, pig, ox, frog) or different regions of the gastrointestinal tract (small intestinal, colonic, gastric, rectal epithelium) of the same animal (6). Again, the layer covering the epithelium varies in thickness and in-

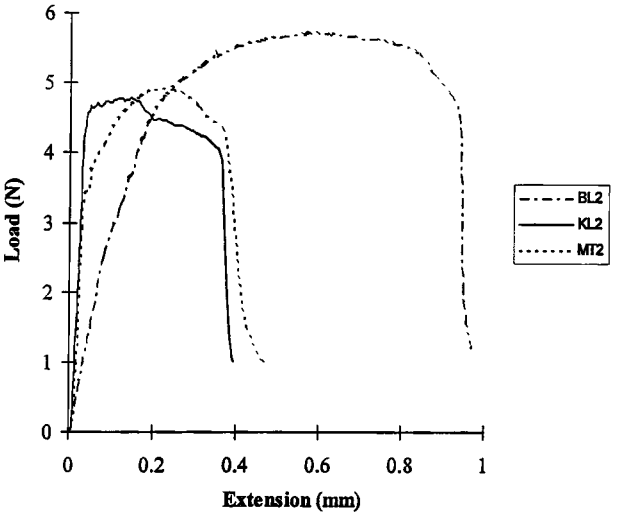

Figure 2. Force versus extension curves for three formulations containing gelatin/NaCMC (BL2), gelatin/HPC (KL2), and gelatin/HPMC (MT2) at a 2:1 ratio.

Table 2

Observed Adhesion with Eggshell Membrane for the Studied Formulations (Without Drugs) (n = 10)

Formulations	Detachment Force (*N*) (Mean ± SD)	Work of Adhesion (mJ) (Mean ± SD)
MT1	3.47 ± 0.69	0.59 ± 0.10
MT2	4.11 ± 0.30	0.68 ± 0.07
MT4	4.34 ± 1.03	0.71 ± 0.27
KL1	4.97 ± 0.75	0.67 ± 0.15
KL2	4.58 ± 0.65	0.59 ± 0.08
KL4	5.08 ± 0.61	0.79 ± 0.09
BL1	5.71 ± 0.35	2.28 ± 0.77
BL2	6.33 ± 0.47	2.55 ± 0.42
BL4	6.15 ± 0.99	1.68 ± 0.66

Table 3

Observed Adhesion with PP Membrane for the Studied Formulations (Without Drugs) (n = 6)

Formulations	Detachment Force (*N*) (Mean ± SD)	Work of Adhesion (mJ) (Mean ± SD)
MT1	4.42 ± 0.39	1.17 ± 0.11
MT2	5.08 ± 0.53	1.57 ± 0.16
MT4	5.22 ± 1.38	0.73 ± 0.37
KL1	5.92 ± 0.38	1.38 ± 0.17
KL2	6.33 ± 0.38	1.59 ± 0.51
KL4	6.51 ± 0.37	1.65 ± 0.34
BL1	4.44 ± 0.30	2.90 ± 0.35
BL2	5.95 ± 0.55	3.18 ± 0.31
BL4	5.94 ± 1.10	2.40 ± 0.68

fluences the adhesive force: the more mucus there is present, the weaker is the adhesive force, which is consistent with the lubricant role of mucus within the gastrointestinal tract (7). This wide variability of the biological materials induced some authors to use synthetic substrates as model membranes (8–10).

The eggshell membrane, employed in this study as a natural substrate, is composed of a matted network of fibers with chemical properties that are intermediate between keratin and chitin, and it presents a standard, smooth, and well-characterized surface (11). Furthermore, this material is more available and easier to handle than animal tissues or mucous membranes.

When this membrane was used, the BL blends (Table 2) showed significantly greater adhesive force than the other polymeric mixtures ($p < .05$) at each considered polymeric ratio, and large peaks were usually recorded (Fig. 2). The MT and KL formulations generally showed lower and similar values. The work of adhesion and the detachment force gave the same information for bond strength, with the relative rank orders of adhesiveness being similar. The excellent bioadhesive properties obtained from the gelatin/NaCMC blends are consistent with the findings reported by several authors (12,13) regarding NaCMC. This cellulose derivative provides the initial hydration and hence the good bioadhesion of the tablet due to its rapid swelling. The presence of gelatin contributes to the adhesiveness of these drug delivery systems and allows to them to maintain their integrity, delaying the erosion time. The presence of HPC and HPMC reduced significantly ($p < .05$) the adhesive force in accordance with the data reported by Çelebi and Kislal (14).

Using a PP membrane (Table 3), the detachment force was significantly ($p < .05$) affected by the proportion of the polymeric blends. The greatest values were observed for the KL formulation at a 1:1 ratio and for KL and BL formulations at a 2:1 ratio. At a 4:1 ratio, no significant differences were observed. On the contrary, BL formulations showed the greatest values of adhesion work at each considered ratio. For this reason, the detachment force and the work of adhesion should not be used interchangeably when comparing mucoadhesion results obtained using the synthetic membrane.

On this basis, the eggshell membrane seems to supply more reliable values of bioadhesion parameters than the synthetic membrane. The use of the egg membrane not only allows taking advantage of biological material, but also avoids the wide variability of several tissues commonly employed in bioadhesion studies. This substrate, therefore, might be a good starting point for future investigations of new natural materials.

The data reported in Table 4 indicated that the rank order of adhesiveness of 2:1 polymeric blends was not modified by the presence of the two drugs.

In Vitro Drug Release Studies

In vitro drug release studies were carried out on the 2:1 gelatin/polymer mixtures. The drug release profiles for BNZ and MTZ are separately reported in Fig. 3 and Fig. 4, respectively. The release rate for both drugs is significantly affected by the polymer employed in the adhesive mixture. The greatest values were observed for the blend containing NaCMC, followed by formulations based on HPC and HPMC, respectively, in accordance with the swelling properties of these polymers.

The release mechanism of the drugs can be analyzed by the following commonly used exponential equation:

$$M_t/M_\infty = kt^n \quad (1)$$

Table 4

Values of Adhesion Obtained with Eggshell Membrane and PP Substrate for the Formulations with Drugs

	Eggshell Membrane ($n = 10$)		PP Membrane ($n = 6$)	
Formulations	Detachment Force (N) (Mean ± SD)	Work of Adhesion (mJ) (Mean ± SD)	Detachment Force (N) (Mean ± SD)	Work of Adhesion (mJ) (Mean ± SD)
BL2D	5.52 ± 0.28	1.88 ± 0.24	5.67 ± 0.29	2.31 ± 0.57
MT2D	3.32 ± 0.73	0.55 ± 0.11	4.61 ± 0.32	0.73 ± 0.17
KL2D	4.05 ± 0.45	0.60 ± 0.11	5.69 ± 0.47	0.97 ± 0.29

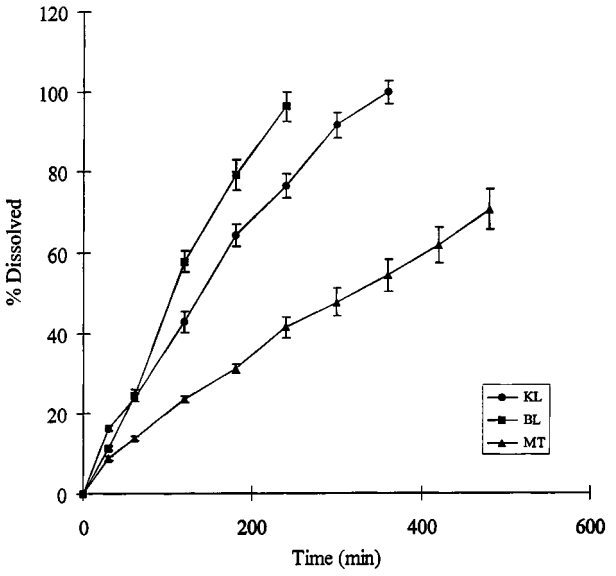

Figure 3. BNZ release profiles (mean values ± SE) in the presence of MTZ.

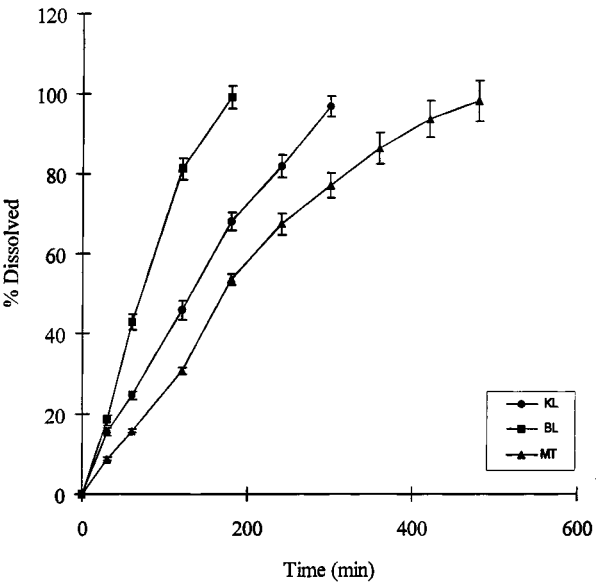

Figure 4. MTZ release profiles (mean values ± SE) in the presence of BNZ.

where M_t/M_∞ is the fraction of the drug released at time t, k is a release constant incorporating structural and geometric characteristics of the drug/polymer system, and n is a release exponent indicative of the release mechanism. When $n = 0.5$, the drug is released from the polymer with a Fickian diffusion mechanism, while for $0.5 < n < 1$, a non-Fickian solute diffusion is observed. The case of $n = 1$ provides a case II transport mechanism with zero-order kinetics (15). The calculated values of n for the release of BNZ and MTZ from the studied formulations are shown in Table 5. These values, ranging from 0.65 to 0.86, were indicative of an ''anomalous or non-Fickian'' mechanism of release that coupled the gradual erosion of the polymeric matrix with the diffusion of the drug. This behavior is dependent on the swelling properties of the used polymers, which also produced the slow dissolution of the systems.

The very similar release rates of BNZ and MTZ from all tested formulations make it possible to combine the two drugs in the same dosage form.

ACKNOWLEDGMENT

This work was partially supported by a MURST grant (Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Rome, Italy).

Table 5

Values of k *and* n *Parameters Estimated by Equation 1*

	Benzydamine		Metronidazole	
Formulations	$k \pm$ SE	$n \pm$ SE	$k \pm$ SE	$n \pm$ SE
MT2D	0.16 ± 0.01	0.66 ± 0.04	0.20 ± 0.01	0.80 ± 0.04
KL2D	0.30 ± 0.01	0.65 ± 0.06	0.31 ± 0.02	0.68 ± 0.06
BL2D	0.29 ± 0.02	0.86 ± 0.06	0.45 ± 0.02	0.75 ± 0.04

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